JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

PROBABLE NOVEL PROBIOTICS: EPS PRODUCTION, CHOLESTEROL REMOVAL AND GLYCOCHOLATE DECONJUGATION OF *LACTOBACILLUS PLANTARUM* GA06 AND GA11 ISOLATED FROM LOCAL HANDMADE-CHEESE

Gulcin Alp Avci[1*], *Gamze Cagatay*[1], *Gizem Ozluk Cilak*[2], *Emre Avci*[1]

Address(es):
[1]Faculty of Science and Arts, Department of Molecular Biology and Genetics, Hitit University, Corum, Turkey.
[2]Faculty of Engineering, Department of Food Engineering, Hitit University, Corum, Turkey.

*Corresponding author: alp.gulcin@yahoo.com , gulcinalp@hitit.edu.tr

doi: 10.15414/jmbfs.2020.10.1.83-86

ARTICLE INFO

Received 11. 11. 2019
Revised 15. 3. 2020
Accepted 23. 3. 2020
Published 1. 8. 2020

Regular article

OPEN ACCESS

ABSTRACT

In this study, it was aimed to investigate the effect of exopolysaccharide produced by 2 novel probiotic strains (*Lactobacillus plantarum* GA06 and GA11), isolated from local handmade cheese, on cholesterol mechanism and bile deconjugation. These two strains were thought to play an important role in cholesterol removal and may contribute to intestinal microbiota positively. The bacterial 16S rRNA gene sequencing analysis was used for molecular identification of the strains. Exopolysaccharide production of the strains was identified by the phenol-sulphuric acid method whereas cholesterol removal and glycocholate deconjugation activity were evaluated via spectrophotometric methods. The exopolysaccharide production of *L. plantarum* GA11 and GA06 was determined as 96.5 and 32.5 mg/L, and the cholesterol removal amounts were found as 36.7% and 28.6% respectively. Glycocholate deconjugation activity of GA11 was detected higher than of GA06. A statistically significant difference was observed between the EPS production capacity of the strains and the removal of cholesterol ($p<0.05$). And also, a statistically significant correlation was found both between the EPS production capacity- cholesterol removal and between EPS production capacity-glycocholate deconjugation. According to the results of our study, *L. plantarum* GA11 strain appears to be a good and novel probiotic strain that has high probiotic potential. Further studies can be confirmed to determine specific more crucial health benefits.

Keywords: Novel probiotics, *L. plantarum,* EPS production, Cholesterol removal, Glycocholate deconjugation

INTRODUCTION

Trillions of microorganisms play a major role in human health and disease. In particular, intestines are seen as “a basic living area” for many of these microorganisms. There are numerous studies indicating that intestinal microbiota is important for human beings. Most of the chronic diseases are emphasized the importance of human microbiota. Probiotics have protective effects on the development of lactose intolerance, cholesterol-lowering, immune system supportive, antibacterial, intestinal infections prevention, intestinal flora and digestive mechanism (**Ley *et al*., 2006; Ursel *et al*., 2014; Kumar and Chordia, 2017; Wang *et al*., 2017**).

For the production of probiotic microorganisms industrially or naturally present in foods, there are some criteria defined by LABIP (Lactic Acid Bacteria Industrial Platform) in order to promote the survival of the probiotics in the digestive system (**Ewaschuk and Dieleman, 2006**). Firstly, probiotic microorganisms; should be of human origin, resistance to digestive enzymes and bile salts, and have antimicrobial activity (**Ooi and Liong, 2010**). Apart from those, one of the desirable characteristics for an organism to be selected as a probiotic is the ability of bile salts deconjugation. This is an important issue for individuals associating with coronary heart disease having high cholesterol levels in the serum (**Lim *et al*., 2004**). Recent studies have reported that blood cholesterol levels have remarkably decreased in individuals who are interested in dieting with probiotic foods including *L. plantarum*, *B. bifidum* and *L. bulgaricus* (**Roos and Katan, 2000; Ooi and Liong, 2010; Zanotti *et al*., 2015**).

Two main hypotheses for cholesterol removal mechanisms of probiotic microorganisms have been proposed which are bile salts deconjugation via the bile salt hydrolase (BSH) enzyme and cholesterol assimilation in the cell membrane (**Noriega *et al*., 2006; Jarocki *et al*., 2014; Sedlackova *et al*., 2015**). Deconjugation of bile acids is thought to help reducing serum cholesterol levels via decreasing the amount of bile acid that is returned to the liver by the enterohepatic cycle so that increasing the production of bile acids via using cholesterol as substrate. This hydrolysis taking place during the enterohepatic circulation is catalyzed by the BSH enzyme produced through intestinal microbiota such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Bacillus*, *Clostridium*, *Fusobacterium*, *Bacteroides*, *Pseudomonas* and *Peptostreptococcus* (**Floch, 2002; Ooi and Liong, 2010).**

Cholesterol assimilation by probiotic microorganism is the second main mechanism thereby reducing lipids and cholesterol levels. It was reported that cholesterol reduction in media was caused by the acidic environment as a result of bile salts decomposition, cholesterol is precipitated with free bile acids, not metabolized by the microorganisms (**Klaver and Van der Meer, 1993; Shehata *et al*., 2016**). Further studies are required to elucidate the exact mechanism(s) of the molecular basis on cholesterol lowering especially on humans.

Sodium glycocholate is the predominant component of bile salts in the human intestine, therefore, the strains that deconjugate sodium glycocholate may be more effective in lowering serum cholesterol (**Choi *et al*., 2015; Mahmoudi *et al*., 2017**).

It was observed in the previous studies that there is a correlation between the EPS production of the probiotic strains and the cholesterol removing mechanism. It was suggested that these polysaccharides may have a similar effect to dietary fiber foods if the amount of EPS produced by the cells is high and thus the cholesterol can be increased by binding to the EPS produced by the bacteria to increase the amount of cholesterol excreted by the bacteria, thus reducing the amount of cholesterol that is absorbed from the intestine into the blood (**Ooi and Liong, 2010; Ayyash *et al*., 2018; Bhat and Bajaj, 2018**).

In this study, it was aimed to investigate the effect of exopolysaccharide, produced by *Lactobacillus plantarum* GA06 and GA11 strains, on cholesterol mechanism and bile deconjugation. These two strains were thought to play an important role in cholesterol removal and may contribute to the intestinal microbiota positively.

MATERIALS AND METHODS

Isolation and culture conditions of Lactobacillus spp.

Lactobacillus spp. were obtained by classical cultivation methods from local handmade-cheese. De Man Rogosa and Sharpe (MRS, Merck-Germany) media were used in the growth and isolation of bacteria. After incubation at 37°C for 18-24 h, the probable *Lactobacillus* spp. colonies were detected by Gram reaction and colony morphology. All strains were activated two times before the usage for all tests.

Biochemical and molecular identification of Lactobacillus spp.

All of the strains were characterized based on the fermentation of carbohydrates using API 50CHL kit (Biomerieux, France). According to color change, the results were evaluated as positive or negative. The results were identified by using the database (V5.1) and apiweb^{TM} identification software.
For the molecular identification of microorganisms, 16S rRNA analysis was used. Firstly, DNA was extracted by Invitrogen Genomic DNA Extraction Kits (Thermo Fisher Scientific) from overnight cultures of pure LAB strains in 5 ml MRS broth and centrifuged at 12000 ×g for 2 min at 4 °C. 16S rRNA gene was amplified by polymerase chain reaction (PCR) to identify isolated bacteria by molecular methods. The universal primers were used 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGTTACCTTGTTACGACTT-3′) for 16S rDNA. The PCR protocol: 94°C 90 s, 48°C 35 s, 50°C 35 s, 72°C 105 s, 33 cycles and a final extension step at 72°C for 3 min, 4°C for 24 h. Sequencing was made via Applied Biosystems BigDye terminator cycle sequencing version 2.0 kit (Applied BioSystems, Foster City, Calif.). Products were purified with Sephadex spin columns (Cold Spring Harbor Protocol, 2002, California), resolved with DNA sequencing system (Applied Biosystems, 3130XL).

EPS production capacity

EPS extraction was performed according to Valerie et al., (1999) 's method. Activated isolates of *Lactobacillus* spp. were firstly adjusted to OD600 nm = ~ 0.600 and inoculated 2 % in MRS medium and incubated at 42°C in anaerobic conditions for 24 hours. After 24 hours, 1 mL of culture was boiled at 96°C for 10-15 minutes and then allowed to cool to room temperature. 17 % of the sample on 1 mL sample was centrifuged for 13 min at 13 000 rpm with 85 % TCA. The supernatants were mixed with an equal volume of ethanol, then centrifuged again. Samples were dissolved by adding ethanol to the pellets for the second time. The dissolved samples were again centrifuged to precipitate. Presipited samples were dissolved in 1 ml dH_2O and the amount of EPS was determined via penol-sulphiric acid method determined by Dubois et al., (Dubois et al**.,1956**). Glucose was used for the standardization of EPS studies (Fig. 1).

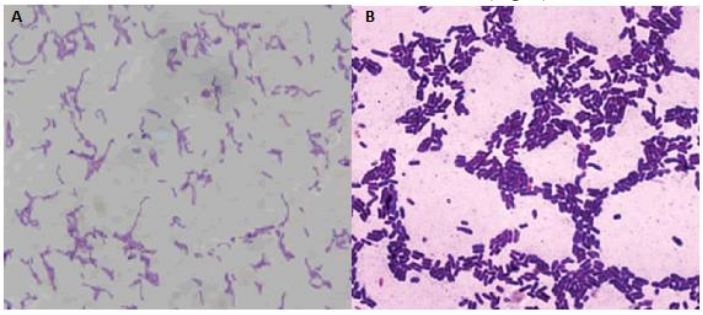

Figure 1 *L. plantarum* GA06 (A) and GA11 (B) microscopy.

Cholesterol removal

A previously modified method by **Alp Avci (2016)** was used for the removal. Cholesterol solution, freshly prepared with ethyl alcohol, was added to MRS broth up to 100 µg/mL final concentrations. These solutions were inoculated as 2% into the media for each strain and incubated at 37 °C for 16-18 h. Bacterial cells were separated via centrifugation at 10.000 g for 20 min, cholesterol in the supernatant was spectrometrically measured at 600 nm. Then they were dried at 80 °C and dissolved in distilled water. Subsequently, the cholesterol assay as described by **Gilliland *et al*., (1985)** was performed. The percentage of assimilations was calculated by the equation established as below;

$$Cholesterol\ assimilation\ (\%) = 100 - \frac{\text{cholesterol amount in inoculated medium}}{\text{cholesterol amount in control medium}}\ x\ 100$$

Deconjugation of sodium glycocholate

The MRS mediums were supplemented with 2 mg/ml sodium glycocholate. Strains were inoculated by 1% (except control groups), incubated for 18-20 h at at same temparature. Based on measuring free cholic acid amount unleashed through each bacterium, deconjugation ability was detected as spectrofotometrically (Abs:660nm, BiochromeLibra, UK) (**Alp Avci, 2016**).

Statistical analysis

Statistical analysis was applied using the SPSS 22.0 software (SPSS Inc., USA). Data are reported as means±SD. The significance level was set at 0.05. Normality distribution was assessed by Shapiro-Wilk test. The non-parametric Kruskal Wallis H-test was applied for comparison of differences between means. In order to find the association between the parameters that were studied, Spearman correlation coefficient was used.

RESULTS AND DISCUSSION

Certain strains of LAB are able to synthesize exopolysaccharides which play a crucial role in the food industry as emulsifying, gelling and stabilizing, moisture-retaining and thickening agents (**Duboc and Mollet, 2001; Jolly *et al*., 2002; Ahmad *et al*., 2015, Venugopal, 2019**). EPS has been claimed to have an anti-tumor effect on human health, immune activating and cholesterol-lowering effect. Therefore, EPS has great potential for its application even in the pharmaceutical industry (**Chun-lei *et al*., 2014**). Moreover, T**ok and Aslim (2010)** suggested that bacterial EPS interacts with the cholesterol in the environment and binds it like dietary fiber.
In this study, the effect of exopolysaccharide that produced by *L. plantarum* GA06 and GA11 strains on cholesterol mechanism was investigated. The strains were thought to play a major role in cholesterol removal and contribute intestinal microbiota positively. In our study, the amount of EPS production through *L. plantarum* GA06 and GA11 was found 32.50 and 96.45 mg/L, respectively (Fig.3). When similar studies in the world are examined, it is seen that EPS production is important for probiotic microorganisms. *L. plantarum* (HQ259238) isolated from Traditional-Chinese fermented foods had high EPS production capability (859 mg/L) (**Feng *et al*., 2012**). EPS amounts of *L. plantarum* YO175 and OF101, which were obtained from Nigerian traditional fermented cereal gruel 'ogi' and grown on sucrose modified mMRS, were found 1.36 g/L and 2.18 g/L, respectively (**Adesulu-Dahunsit *et al*., 2018**). **Khalil *et al*., (2018)** found that EPS production, via *L. plantarum* TEMP9 strain obtained from traditional Malaysian fermented foods, was 80 mg/L, EPS yield of *L. fermentum* PIC7 was 750 mg/L. **Van Der Meulen *et al*.**, (2007) reported that EPS amounts of ten *Lactobacillus* strains isolated from dairy and cereal products, were ranged from 0.8 and 1.7 g/ L. EPS levels of *L. plantarum* NTMI05 and NTMI20 were observed as 250 mg/L by **Imran *et al*., (2016)**. **Ismail and Nampoothiri (2010**) found that the *L. plantarum* MTCC 9510, produce 210 mg/L EPS in media (added 2% sucrose). In previous work, yield of EPS from *L. plantarum* MCC2034 shows differences in MRS broth with glucose, mod-MRS broth with sucrose and synthetic medium with lactose producing 251 mg/L, 1.059 mg/L, and 1.060 mg/L, respectively. Therefore, it could be suggested that EPS production levels can depend on carbon source, sugar carbohydrate types in the culture medium, and bacterial strain (**Badel *et al*., 2011**). **Seo *et al*. (2015)** isolated *L. plantarum* YML009 on MRS supplemented with 10% glucose, producing 260 mg/L EPS. The media components have affected EPS production because of especially carbohydrate/carbon sources in the medium.

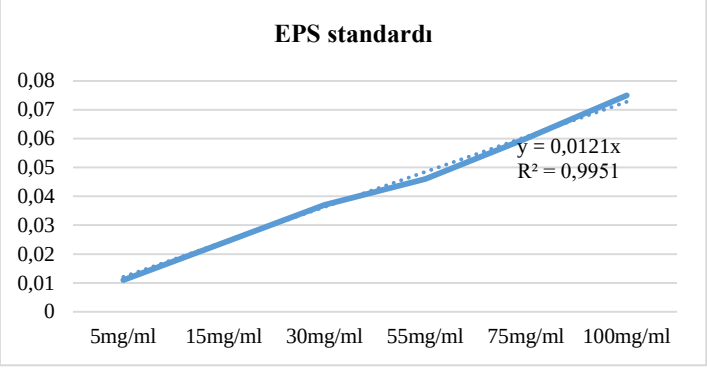

Figure 2 The standard EPS graphic that prepared with glucose.

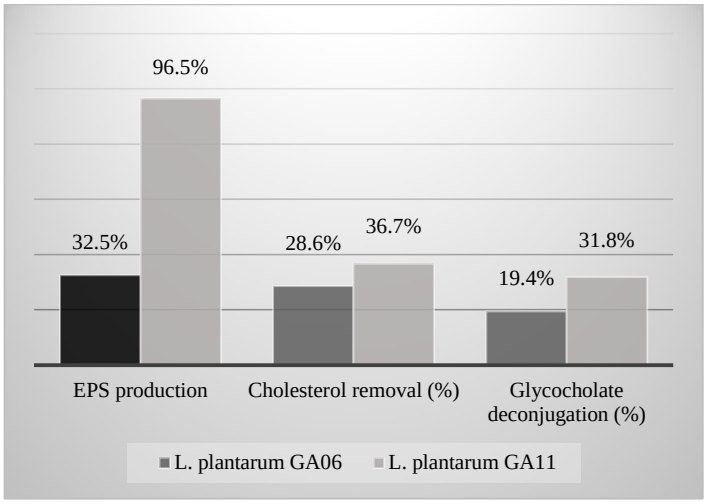

Figure 3 EPS production, cholesterol removal, and glycocholate deconjugation values of *L. plantarum* GA06 and GA11

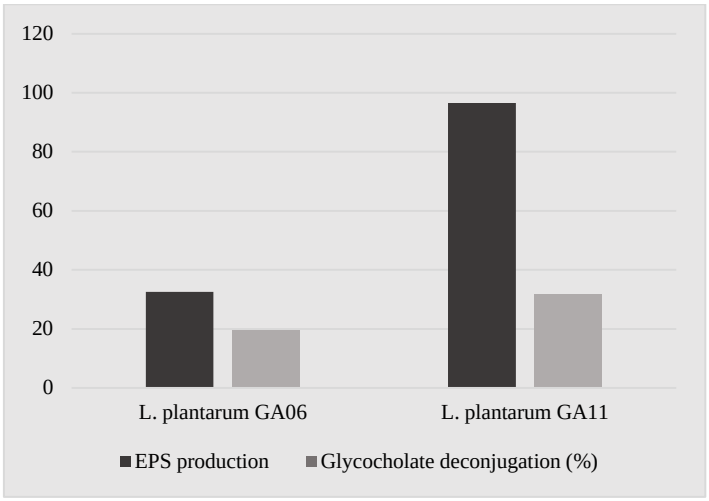

Figure 4 EPS production-glycocholate deconjugation values of *L. plantarum* GA06 and GA11

Cholesterol is a necessary lipid derivative for all living things. This substance is a major component of the cell membrane, bile acids, steroid hormones and the precursor molecule of vitamin D and has an important role in metabolic activities. Until now, some studies have shown that probiotics like *L. acidophilus, B. bifidum*, and *L. bulgaricus* significantly reduce cholesterol levels in coronary heart disease (**Ooi and Liong, 2010**). Lowering blood cholesterol levels have been increasing because of probiotics usage. **Ngongang *et al*., (2016**) observed two of three isolates from palm wine were *L. plantarum* which were able to decrease cholesterol level in MRS broth ($p<0.05$). *L. plantarum* 2R30 strain assimilated 51.25% cholesterol in the media while *L. plantarum* 2R36 had 55.22% cholesterol reduction. *L. plantarum* and *L. paracasei* isolated from cheese were investigated in-vitro to decrease cholesterol (Belviso et al., 2009). *L. plantarum* and *L. paracasei* showed 19.4 % and 6.8 % cholesterol removal, respectively. Guo et al. (2011) found 23.55- 54.08 % cholesterol removal ability of 6 *L. plantarum* strains obtained from home-made fermented cream in Mongolia, China. In our study, the cholesterol removal ability of *L. plantarum* GA06 was 28.56 % while *L. plantarum* GA11 has 36.57 % (Fig. 3). A significant difference was observed between EPS production and cholesterol removal ($p<0.05$). And also, a statistically positive correlation was determinated between EPS production and cholesterol removal (Fig. 4). Most of the studies reported that there is a correlation between the EPS production of the probiotic strains and the cholesterol removing mechanism, so our research showed parallelism to this situation. Compared with the studies, *L. plantarum* has an average EPS production which might result in average cholesterol removal.

Anandharaj and Sivasankari (2014) investigated the deconjugation ability of isolated *L. oris* strains from mother's milk. HMI68 strain had the highest deconjugation ability for taurocholate (0.98 mM) and glycocholate (0.89 mM). In another research studied with *B. pseudocatenulatum* G4, which is thought as a probable probiotic strain, was isolated from infant feces samples. It showed to deconjugation of all bile salts at the level of 0.25 mM at pH 5.7 **(Rasti *et al*., 2011)**. In our study, the glycocholate deconjugation capacity of *L. plantarum* GA06 and GA11 strains were 1.02±0.1 mM (19.42%) and 1.30±0.2 mM (31.84%), respectively (Fig. 3). Compared with the previous studies, *L. plantarum* GA11 has a dramatically high concentration of glucocholate deconjugation. Since the strains which deconjugate sodium glycocholate may be more effective in lowering serum cholesterol (**Mahmoudi *et al*., 2017**), *L. plantarum* GA11 could have more hypocholesteromic effect in order to prevent hypercholesterolemia. A statistically significant difference was found between the EPS production and glycocholate deconjugation ($p<0.05$). Moreover, a statistically significant correlation was found between EPS production and deconjugation.

CONCLUSIONS

Today, many probiotic microorganisms are discovered, however, it is important that these microorganisms are probiotic microorganisms with superior characteristics. In our study, cholesterol removal and glycocholate deconjugation of the strains were evaluated together in order to reveal the superior probiotic microorganisms. *L. plantarum* GA11 strain showed important results in the EPS production level, cholesterol removal and high concentration of glycocholate deconjugation in this study. According to the results of our study, *L. plantarum* GA11 strain appears to be a good and novel probiotic strain that has high probiotic potential. Further studies can be confirmed to determine more crucial health benefits.

Funding sources: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Adesulu-Dahunsı, A.T., Jeyaram, K., Sannı, A.I., & Banwo, K. (2018). Production of exopolysaccharide by strains of *Lactobacillus plantarum* YO175 and OF101 isolated from traditional fermented cereal beverage. *Peer Journal*, 6, E5326. http://dx.doi.org/10.7717/peerj.5326

Ahmad, N.H., Mustafa, S., & Man, Y.B.C. (2015). Microbial polysaccharides and their modification approaches: A Review. *International Journal of Food Properties*, 18(2), 332-347. http://dx.doi.org/10.1080/10942912.2012.693561

Avcı, G.A. (2016). Selection of superior bifidobacteria in the presence of rotavirus. *Brazilian Journal of Medical and Biological Research*, 49(11), E5562. http://dx.doi.org/10.1590/1414-431x20165562

Anandharaj, M., & Sıvasankarı. B. (2014). Isolation of potential probiotic lactobacillus oris hmı68 from mother's milk with cholesterol-reducing property. *Journal of Bioscience and Bioengineering*, 118(2), 153-159. http://dx.doi.org/10.1016/j.jbiosc.2014.01.015

Ayyash, M., Abushelaibi, A., Al-Mahadin, S., Enan, M., El-Tarabily, K., & Shah, N. (2018). In-vitro investigation into probiotic characterization of *Streptococcus* and *Enterococcus* isolated from camel milk. *LWT- Food Science and Technology*, 87, 478-487. http://dx.doi.org/10.1016/j.lwt.2017.09.019

Badel, S., Bernardı, T., & Mıchaud, P. (2011). New perspectives for lactobacilli exopolysaccharides. *Biotechnology Advance*, 29, 54–66. http://dx.doi.org/10.1016/j.biotechadv.2010.08.011

Belvıso, S., Gıordano, M., Dolcı, P., & Zeppa, G. (2009). In vitro cholesterol-lowering activity of *Lactobacillus plantarum* and *Lactobacillus paracasei* strains isolated from the Italian Castelmagno PDO cheese. *Dairy Science and Technology*. 89, 169–176. http://dx.doi.org/10.1051/dst/2009004

Bhat, B., & Bajaj, B.K. (2018). Hypocholesterolemic and bioactive potential of exopolysaccharide from a probiotic *Enterococcus faecium* K1 isolated from kalarei. *Bioresource Technology*, 254, 264-267. http://dx.doi.org/10.1016/j.biortech.2018.01.078

Chun-Leı, Z., Jıa-Qı, L., Haı-Tao, G., Jıe, W., & Rıhua, X. (2014). Selection of exopolysaccharide-producing lactic acid bacteria isolates from Inner Mongolian traditional yoghurt. *Mljekarstvo Dairy*, 64(4), 254-260. http://dx.doi.org/10.15567/mljekarstvo.2014.0404

Choı, S.B., Lew, L.C., Yeo, S.K., Parvathy, S.N., & Lıong, M.T. (2015). Probiotics and the BSH-related cholesterol lowering mechanism: A Jekyll and Hyde scenario. *Critical Reviews in Biotechnology*, 35(3), 392-401. http://dx.doi.org/10.3109/07388551.2014.889077

Duboc, P., & Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal*, 11, 759-768. http://dx.doi.org/10.1016/s0958-6946(01)00119-4

Duboıs, M., Gılles, K.A., Hamılton, J.K., Rebers, P.A., & Smıt, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356. http://dx.doi.org/10.1021/ac60111a017

Ewaschuk, J.B., & Dıeleman, L.A. (2006). Probiotics and prebiotics in chronic inflammatory bowel diseases. *World Journal of Gastroenterology*, 7(12), 5941-5950. http://dx.doi.org/10.3748/wjg.v12.i37.5941

Feng, M., Chen, X., Lı, C., Nurgul, R., & Dong, M. (2012). Isolation and identification of an exopolysaccharide-producing lactic acid bacterium strain from Chinese Paocai and biosorption of PB(II) by its exopolysaccharide. *Journal of Food Science*, 77(6), 111-117. http://dx.doi.org/10.1111/j.1750-3841.2012.02734.x

Floch, M.H. (2002). Bile salts, intestinal microflora and enterohepatic circulation. *Digestive and Liver Disease*, 34, 54–57. http://dx.doi.org/10.1016/S1590-8658(02)80165-7

Gilliland, S.E., Nelson, C.R., & Maxwell, C. (1985). Assimilation of cholesterol by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, 49(2), 377-381. http://dx.doi.org/10.0099-2240/85/020377

Guo, L.D., Yang, L.J., & Huo G.C. (2011). Cholesterol removal by *Lactobacillus plantarum* isolated from homemade fermented cream in Inner Mongolia of China. *Czech Journal of Food Sciences*, 29(3), 219–225. http://dx.doi.org/10.17221/224/2009-CJFS

Imran, M.Y.M., Reehana, N., Jayaraj, K.A., Ahamed, A.A.P., Dhanasekaran, D., Thajuddın, N., & Alharbı, N.S., Muralıtharan, G. (2016). Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20. *International Journal of Biological Macromolecules*, 93(Pt A), 731-745. http://dx.doi.org/10.1016/j.ijbiomac.2016.09.007

Ismaıl, B., & Nampoothırı, K.M., (2010). Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. *Archives of Microbiology*, 192(12), 1049-1057. http://dx.doi.org/10.1007/S00203-010-0636-y

Jarockı, P., PodleśNy, M., Glıbowskı, P., & Targońskı, Z. (2014). A new insight into the physiological role of bile salt hydrolase among intestinal bacteria from the genus *Bifidobacterium*. *PLOS ONE*, 9(12), 1–24. http://dx.doi.org/10.1371/journal.pone.0114379

Jolly, L., Vıncent, S.J.F., Duboc, P., & Neeser, J.R.. (2002). Exploiting exopolysaccharides from lactic acid bacteria. *Antonie Van Leeuwenhoek*, 82(1-4), 367-374. http://dx.doi.org/10.1023/A:1020668523541

Khalıl, E.S., Manap, M.Y., Mustafa, S., Amıd, M., Alhellı, A.M., & Aljouborı, A. (2018). Probiotic characteristics of exopolysaccharides producing *Lactobacillus* isolated from some traditional Malaysian fermented foods. *CyTA-Journal of Food*, 16(1), 287–298. http://dx.doi.org/10.1080/19476337.2017.1401007

Kıran, F., & Osmanagaoglu, Ö. (2012). The Use of Lactic Acid Bacteria as Probiotic. *Selcuk Journal of Agriculture and Food Sciences.* 26(4), 60-67.

Klaver, F.A.M., & Van-Der-Meer, R. (1993). The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. *Applied and Environmental Microbiology*, 59(4), 1120–1124. http://dx.doi.org/10.1128/AEM.59.4.1120-1124.1993

Kumar, A., & Chordıa, N. (2017). Role of microbes in human health. *Applied Microbiology*, 3(2), 131-133. http://dx.doi.org/10.4172/2471-9315.1000131

Ley, R.E., Peterson, D.A., & Gordon, J.I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*, 124(4), 837-848. http://dx.doi.org/10.1016/j.cell.2006.02.017

Lım, H., Kım, S., & Lee, W. (2004). Isolation of cholesterol-lowering lactic acid bacteria from human intestine for probiotic use. *Journal of Veterinary Science*, 5(4), 391- 395. http://dx.doi.org/10.4142/jvs.2004.5.4.391

Mahmoudı, I., Moussa, O.B., & Hassouna, M. (2017). Symbiotic, hypocholesterolemic and antioxidant effects of potential probiotic lactobacilli strains isolated from Tunisian Camel Milk. *Advences in Microbiology*, 7(04), 328-342. http://dx.doi.org/10.4236/aim.2017.74027

Ngongang, E.F.T., Tıencheu, B., Fossi, B.T., Achidi, A.U., Shiynyuy, D.M., Womeni, H.M., & François, Z.N. (2016). Isolation and identification of cholesterol lowering probiotic bacteria from Palm Wine (Raffia Mambillensis). *Journal of Microbiological Research*, 6(5), 93-102. http://dx.doi.org/10.5923/j.microbiology.20160605.01

Norıega, L., Cuevas, I., Margolles, A., & De Los Reyes-Gavılán, C.G. (2006). Deconjugation and bile salts hydrolase activity by *Bifidobacterium* strains with acquired resistance to bile. International Dairy Journal 16(8), 850–855. http://dx.doi.org/10.1016/j.idairyj.2005.09.008

Ooı, L.G., & Lıong, M.T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. *International journal of Molecular Sciences*, 11(6), 2499-2522. http://dx.doi.org/10.3390/ijms11062499

Rastı, B., Shuhaımı, M., Anıs-Shobırın, M.H., Nateghı, L., Meımandıpour, A., & Mohd-Yazıd, A.M. (2011). Bile resistance and bile salt deconjugation activity of bifidobacterium pseudocatenulatum G4 in a simulated colonic pH. *Journal of Food and Agricultural Environmental*, 9(1), 89-94. EID: 2-s2.0-80755155816

Roos, N.M., & Katan, M.B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. *The American Journal of Clinical Nutrition*, 71, 405-411. http://dx.doi.org/10.1093/ajcn/71.2.405

Sedlackova, P., Horackova, S., Shi, T., Kosova, M., & Plockova, M. (2015). Two different methods for screening of bile salt hydrolase activity in *Lactobacillus* strains. *Czech Journal of Food Sciences*, 33(1), 13–18. http://dx.doi.org/10.17221/299/2014-CJFS

Seo, B.J., Bajpai, V.K., Rather, I.A., & Park, Y.H. (2015). Partially purified exopolysaccharide from lactobacillus plantarum YMl009 with total phenolic content, antioxidant and free radical scavenging efficacy. *Indian Journal of Pharmaceutical Education and Research*, 49(4), 282-292. http://dx.doi.org/10.5530/ijper.49.4.6

Shehataa, M.G., El-Sohaimy, G.A., El-Sahn, M.A., & Youssef, M.M. (2016). Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Annals of Agricultural Sciences*, 61(1), 65-75. http://dx.doi.org/10.1016/j.aoas.2016.03.001

Tok, E., & Aslim, B. (2010). Cholesterol removal by some lactic acid bacteria that can be used probiotic. *Microbiology and Immunology*, 54(5), 257–264. http://dx.doi.org/10.1111/j.1348-0421.2010.00219.x

Ursell, L.K., Haiser, H.J., Van Treuren, W., Garg, N., Reddivari, L., Vanamala, J., Dorrestein, P.C., Turnbaugh, P.J., & Knight, R. (2014). The intestinal metabolome: an intersection between microbiota and host. *Gastroenterology*, 146(6), 1470-1476. http://dx.doi.org/10.1053/j.gastro.2014.03.001

Wang, B., Ya,O M., Lv, L., Ling, Z., & Li, L. (2017). The human microbiota in health and disease. *Engineering*, 3(1), 71-82. http://dx.doi.org/10.1016/j.eng.2017.01.008

Van Der Meulen, R., Grosu-Tudor, S., Mozzi, F., Vaningelgem, F., Zamfir, M., De Valdez, G.F., & De Vuyst, L. (2007). Screening of lactic acid bacteria isolates from dairy and cereal products for exopolysaccharide production and genes involved. *International Journal of Food and Microbiology*, 118, 250–258. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.014

Venugopal V. (2019). *Applications of marine polysaccharides in food processing in enzimatic technologies for marine polysaccharides* (Ed. Antonio Trincone). Crc Press, Ny. http://dx.doi.org/10.1201/9780429058653-2

Zanotti, I., Turroni, F., Piemontese, A., Mancabelli, L., Milani, C., Viappiani, A., Prevedini, G., Sanchez, B., Margolles, A., Elviri, L., Franco, B., Van Sinderen, D., & Ventura, M. (2015). Evidence for cholesterol-lowering activity by *bifidobacterium bifidum* prl2010 through gut microbiota modulation. *Applied Microbiology and Biotechnology*, 99(16), 6813–6829. http://dx.doi.org/10.1007/S00253-015-6564-7